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Antibiosis of Physical Characteristics of Maize Grains to *Sitotroga cerealella* (Oliv.) (Gelechiidae: Lepidoptera) in Free Choice Test

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Abstract

Physical characteristics of eight maize varieties /lines (EV-6089, Sahiwal-2002, Golden, 34N43, EV-1098, Sultan, China-1, EV-20), seven yellow and one white, obtained from Maize and Millet Research Institute, Yousaf Wala, Sahiwal, Punjab, Pakistan, in free choice test, were correlated with biological parameters of life history of *Sitotroga cerealella* (Oliv.) (Gelechiidae: Lepidoptera). *S. cerealella* was cultured on a susceptible maize variety for two generations and was then transferred on the grains of the test varieties / lines for further experiments.

Results showed that the maximum number of moths was emerged in Sultan (9.33) and China-1 (9.33). Fecundity was highest on variety EV-6089 (50.00). Maximum number of eggs hatched in Sultan (87.83%). Highest moth weight was observed in variety EV-6089 (7.82 mg). Maximum development time was shared by China 1 and 32N43 (32.67 and 32.33 days, respectively). Maximum percent grain damage and weigh loss was in EV-1098 (93.46% and 42.19%, respectively). Average grain weight was maximum (32.33 mg) in China-1. EV-6089 and EV-1098 had significantly high hardness index. The varieties had positive as well as negative correlation between hardness index and average grain weight (1000 grains) and life history parameters. On the basis of correlation, involvement of the grain characteristics in the resistance of maize grain towards *S. cerealella* is discussed.

Key words: resistance, maize grains, physical factors, *S. cerealella*

Introduction

Food grains are stored for short and long period in order to meet the need of the expanding human population and to ensure supply and distribution more or less evenly until next production in the developing countries. During storage, several factors affect quality and quantity of these grains (Chughtai *et al*., 2002; Hussain *et al.,* 2003).

In Pakistan, cereals are stored at the grain moisture content < 10%. This moisture level is sufficient to provide protection against insects and fungi, however, moisture level is increased in storage during rainy season. At this time, insects attack and play havoc with grains. Insecticides are used to protect the grains from insects. These insecticides are used as protectants either in sprayable form or in gaseous form (fumigant). Several indigenous studies have indicated development of resistance to these protectants and fumigants, and the ability of insects to acquire resistance to insecticides has put a question mark on the efficacy of existing and future chemicals (Irshad and Gillani, 1988; Iqbal and Irshad, 1993).

In an attempt to reset the integrated pest management of the insects of stored grains, first line of defence is usually considered by natural resistance in grains to ward off insects pests. Grain varieties differ in their attributes to resist insect pests. Biochemical factors of resistance have been identified; nevertheless, morphological and physical factors of the grains substantiate the property of resistance against insects. Both factors may act synergistically in several wheat, rice and maize/corn varieties to protect grains against *Tribolium castaneum*, *Rhizopertha dominica*, *Trogoderma granarium, Sitophilus* spp., *Plodia interpunctella* and *Sitotroga cerealella* (Khattak *et al*., 1996 a, b; Ahmed *et al*., 2002; Shafique and Chaudry, 2007). Resistance in stored maize to *S. cerealella* and *Sitophilus zeamais* attack has been attributed to a number of factors including kernel hardness (Dobie, 1974; Serratos *et al*., 1987), husk protection (Dobie, 1977), obstruction from adjacent kernels (Kossou *et al*., 1992) and kernel size and texture (Kossou *et al*., 1993). The physical characters of maize grains are less focused as compared to biochemical characters that confer the susceptibility of the maize grains towards *S. cerealella*. (Wahla *et al*., 1984; Shazali, 1987; Khattak *et al.*, 1988; Hamed and Khan, 1994; Shazali, 1997; Aslam *et al*., 2004; Shafique *et al*., 2006). The present studies were carried out to determine effect of morphological and physical characteristics of maize grain on biology of *Sitotroga cerealella* in free choice test.

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Materials and Methods

The experiments were conducted in the Toxicology Laboratory, Department of Agricultural Entomology, University of Agriculture Faisalabad. The temperature and humidity was maintained at 30^0C±5 and 65±5 % RH in the rearing room.

Maize varieties: Eight maize varieties / lines, seven yellow and one white, were obtained from Maize and Millet Research Institute, Yousaf Wala, Sahiwal, Punjab. Freshly laid eggs (one day old) of *S. cerealella* were obtained and cultured on a susceptible maize variety for two generations. All test maize varieties / lines were conditioned in the laboratory for at least twenty days before releasing the adults on them.

Experiments on biology of *S. cerealella*

Free choice chamber: Free choice chamber was made up of thermopore (18×18cm^2). The area of chamber was divided into 10 equal parts and 20 gm sample of each variety was placed in each part. The chamber was covered on the top with transparent polythene sheet. 20 pairs of 1-2 day old adult of *S. cerealella* were released from upper opening and opening was closed by a piece of paper tape. The insect was allowed freely to oviposit in any of test variety for seven days. After seven days the entire samples with eggs of *S. cerealella* (Oliv.) were shifted in 275 ml jars after removing dead adults and this was repeated three times.

Developmental Time (DT): 20 gm sample of each variety was placed in the plastic jars of 275 ml capacity and 50 one day old eggs of *S. cerealella*, after separating under binocular, were inoculated. The mouths of the jars were covered with muslin cloth. Growth period was measured by recording the time between inoculations to adult emergence (in days) in each test variety for two successive generations.

Adult Emergence: Percent adult emergence was calculated from eggs inoculated and adult emerged from these and are represented in percent.

Adult weight: The newly emerged adults from each sample were weighed on an electronic balance.

Fecundity / Number of eggs: Fecundity was studied on each variety in two generations by transferring newly emerged adults (1 male and 1 female) of *S. cerealella* into plastic vials (6.5 × 4 cm). Black paper strips were used to collect the eggs as described by Consoli and Filho (1995). The paper strips were removed daily and numbers of eggs were recorded until the death of the females.

Egg hatching period and percentage: The egg hatching period was studied by collecting eggs of each generation from each treatment. A sample of 50 eggs from each treatment was pasted on paper strip and these strips were placed in the jars. Paper strips were removed daily from jars and the number of egg hatched was recorded. Hatching period was noted as interval in days from pasting time to 1st instar larval emergence. The percentage of egg hatching was calculated by counting number of larvae from 50 eggs and expressed in percent.

The biological parameters were studied in the experiments laid out as Completely Randomized Design and were repeated three times.

Percentage Damage: The entire insect infested sample was sieved through 60 mesh screen. Dust was discarded and all the sound and damaged grains were separated and weighed. The % damage was calculated by the following formula.

$$\% \text{ Damage} = \frac{\text{weight of control sample} - \text{weight of sound grain sample}}{\text{Weight of control sample}} \times 100$$

Percentage Weight Loss: The entire insect infested sample was sieved through 60 mesh screen. Dust was discarded and all the sound and damage grain were collectively weighed. The % weight loss was calculated by the following formula.

$$\% \text{ Weight Loss} = \frac{\text{weight of control sample} - \text{weight of (sound +damaged) grain sample}}{\text{Weight of control sample}} \times 100$$

Physical Grain characters:

Grain Shape: Grain shape was determined from the description given by the National Guidelines for the Conduct of Test for Distinctness, Uniformity and Stability of Maize (agricoop.nic.in/SeedTestguide/maize.htm). Weight of 1000 grains was recorded on an electronic balance.

Grain Hardness: In a milling method, 20gm samples of the varieties equilibrated at 27°C and 70% RH were placed into the mill hopper and the mill was closed. The mill speed was set at 6000 rpm and allowed to run for 30 s to attain a constant speed. The material emitted from the grinding chamber was collected in the plastic tube. The partially ground material retained within the grinding chamber was also collected using an 80 mm aperture sieve. The weight of the material retained by the sieve and which passed through the sieve, was taken. Each sample was replicated thrice. A hardness index (HI) was then calculated according to the formula:

$$\text{HI} = \frac{\text{Weight of retained part of flour}}{\text{Weight of sieved part of flour}}$$

Grain Bulk Density: The average bulk density was measured by gently filling a 1000 cc container with the grain and then weighing it. The average true

density was determined using the toluene displacement method (Ahmadi *et al*., 2009).
Porosity of husk was computed from the values of true density and bulk density using Equation.
$\varepsilon = [1-(\rho_b/\rho_t)] \times 100$

Where, ε = porosity, per cent; ρ_b = bulk density, kg/m^3, ρ_t = true density, kg/m^3

Specific Gravity: Grains samples for the specific gravity technique were drawn by volume and averaged 20g. These samples were weighed first in air and again when suspended in a mesh basket submerged in tap water plus a wetting agent to maximize contact of seeds with water.
Specific gravity: Weight in air / (weight in air - weight in water)
Statistical Analysis: The data recorded in the experiments were statistically analyzed with Statistica using Least Significant Difference at P<0.05 to separate means.

Results

The moth emergence showed significant differences among the varieties (Table 1). The maximum number of moths emerged in Sultan (9.33) and China-1 (9.33) while minimum in Sahiwal 2002 (5.00) and was significantly different from former two varieties. Variety EV-6089 had maximum number of eggs (50.00) and showed non significant difference with Sultan (49.50). The varieties 34N43 (22.67) Golden (23.50) EV-20 (24.33) and China-1 (25.33) had non significant difference among them for fecundity. The variety EV-1098 had minimum mean number of eggs (11.33). Significant differences existed among the varieties for egg hatching percentage. Maximum egg hatching in Sultan (87.83%) showed non-significant difference with EV-20 (85.16%). Variety China-1 had minimum hatching percentage (59.33%) and was significantly different from other varieties. Highest moth weight in variety EV-6089 (7.82 mg) had significant difference from all other varieties. Variety Golden had minimum mean moth weight (2.87 mg) and had non significant difference with Sultan (3.10 mg), EV-20 (3.05 mg) and China-1 (2.93 mg). Maximum development time was shared by China 1 and 32N43 (32.67 and 32.33 days, respectively) and had non significant difference with Sultan, EV 6089 and EV 1098. Maximum percent grain damage in EV-1098 (93.46%) had non significant difference with Golden (93.07%), Sahiwal-2002 (89.90%), Sultan (88.58%) and EV 20 (87.35%). Variety 34N43 had minimum percentage grain damage (82.46%) and showed significant difference with EV-1098. Maximum percentage weight loss (42.19%) in EV-1098 had significant difference from all other varieties. China-1 had minimum weight loss and was non-significantly different form all varieties except EV-1098 and Sahiwal 2002.
The values of Grain Bulk Density, Porosity of husk, Specific Gravity had stastistical non-significant difference among the varieties, and hence not shown here. Table 2 shows that 1000 grain weight was maximum (32.33 gm) in China-1 and had non significant difference with Sultan, Golden, Sahiwal 2002 and EV-1098. EV-6089 and EV-1098 had significantly high hardness index, however, lowest hardness index (1.67) in Sultan had non significant difference with Golden, Sahiwal 2002, EV-20, China-1 and 34N43.
Coefficient of correlation of grain hardness Index and 1000 grain weight with the life history parameters of *S. cerealella* showed that among the varieties, parameters had positive as well as negative correlation with hardness index and 1000 grain weight (Tables 3 and 4).

Discussion

Resistance and susceptibility of cereal grains to storage insect pests has been studied in terms of damage and weight loss, which were greater on susceptible varieties than resistant varieties. Percent damage and weight loss was high on EV-1098 with lowest developmental time. These results of high percent damage and weight loss in EV 1098 and other varieties are in line with Wahla *et al*. (1984), Aslam *et al*. (2004), Shafique *et al*. (2006) and Shafique and Chaudry (2007). This shows that larvae of *S. cerealella* consumed more grains to develop in short time. These previous studies have also included moth emergence as index of resistance / susceptibility. The number of moth emerged in EV 1089 was significantly less and had non significant difference with varieties which shared the same results. The percent damage and weight in these varieties was nearer to those showing high number of moth emerged. The present results indicate that varieties preference cannot be judged with one criterion, rather many factors should be taken altogether.
The hardness of grains has been regarded as resistance factor in maize and sorghum (Gudrups *et al*. 2001), but contrasting results were obtained in the present studies. Varieties having high hardness index were greatly damaged and could not prolong developmental time. Low fecundity in hard varieties (such as EV-1098) may be due to biochemical factors which may affect the ability of females to lay more eggs. Developmental time was positively correlated with hardness in varieties having low and high

Table 1 Comparison of life history characteristics of *S. cerealella* on different test varieties of maize

Varieties	Emergence %	Fecundity	Hatching %	Weight mg	DT Days	Damage %	Weight loss %
34N43	6.00bc	22.67c	73.66bc	6.05b	32.33ab	82.46c	26.96bc
Golden	8.33ab	23.50c	67.33cd	2.87e	29.66d	93.07a	27.09bc
Sahiwal 2002	5.00c	37.16b	76.83abc	4.17d	31.33bc	89.90ab	31.78b
EV-1098	5.33c	11.33d	68.16cd	4.68c	29.66d	93.46a	42.19a
EV-6089	5.66c	50.00a	76.33abc	7.82a	31.33bc	91.52ab	26.35bc
Sultan	9.33a	49.50a	87.83a	3.10e	31.33bc	88.58abc	28.10bc
EV-20	6.33bc	24.33c	85.16ab	3.05e	30.33cd	87.35abc	28.24bc
China-1	9.33a	25.33c	59.33d	2.93e	32.67ab	85.08bc	23.02c
LSD	1.16	3.81	6.60	1.47	1.47	3.08	3.25

Development time (DT). Values are means of two generations which in a column with same letter are non significantly different among them at P<0.05.

Table 2 Physical Characters of Maize Grains of different varieties

Variety	Colour	Grain Type	1000 grain weight (gm)	Hardness index
34N43	Yellow	Dented	26.05 bc	1.79bc
EV 6089	White	Round	25.19 bc	2.63a
EV1098	Yellow	Round	27.08 abc	2.56a
China-1	Yellow	Dented	32.23 a	1.96bc
EV -20	Yellow	Flint	24.71 c	1.74bc
Sultan	Yellow	Round	26.67 abc	1.67c
Golden	Yellow	Semi dented	30.32 abc	1.95c
Sahiwal 2002	Yellow	Semi dented	26.57 abc	1.87bc

Values are means of two generations which in a column with same letter are non significantly different among them at P<0.05.

Table 3 Comparison of Coefficient of correlation of Hardness Index with life history parameters of *S. cerealella*, weight loss and percent damage in different varieties of maize

Variety	Moth Emergence	No. of eggs	Hatching %	Moth weight	Damage (%)	Weight loss (%)	DT
34N43	-0.93	-0.27	0.08	0.02	0.93	-0.94	0.34
EV-6089	-0.99	0.60	0.89	0.16	-0.56	-0.60	-0.52
EV-1098	0.85	-0.69	-0.53	-0.21	0.43	0.51	0.98
China-1	-0.65	-0.60	-0.84	0.19	0.99	0.60	-0.65
EV-20	-0.70	0.90	0.83	-0.91	-0.83	0.73	0.90
Sultan	-0.33	-0.01	0.96	0.80	0.43	0.28	-0.68
Golden	-0.99	-0.96	0.27	-0.80	0.98	-0.98	-0.80
Sahiwal-2002	0.72	-0.69	-0.64	-0.89	-0.48	0.98	-0.27

Table 4 Comparison of Coefficient of correlation of 1000 grain weight with the life history parameters of *S. cerealella*, weight loss and percent damage in different varieties of maize

Variety	Moth Emergence	No. of eggs	Hatching %	Moth weight (mg)	Damage (%)	Weight loss (%)	DT
34N43	0.94	0.83	-0.70	0.62	-0.40	-0.50	0.34
EV-6089	-0.99	0.60	-0.34	0.85	0.76	0.73	-0.79
EV-1098	0.99	-0.12	0.08	-0.75	0.89	-0.11	0.68
China-1	-0.89	0.95	0.29	-0.90	0.22	0.92	-0.89
EV-20	-0.36	0.65	0.54	-1.0	-0.9	0.40	0.65
Sultan	0.98	-0.91	0.07	-0.73	0.79	0.88	0.84
Golden	0.83	0.92	-0.80	0.27	-0.66	0.87	0.27
Sahiwal-2002	-0.05	-0.99	0.16	0.50	0.99	0.50	0.54

hardness index. Thus hardness alone is not sufficient to impart resistance in grains to *S. cerealella*. It may be true from other insect species such as *Sitophilus* spp., which pierce the outer of grains to insert eggs and then plug it. This is further shown in another example. Sultan with least hardness index was negatively correlated with percent damage and weight loss.

On the basis of results obtained, it can be stated that physical and morphological characters of maize grains may confer resistance in combination with some other factors particularly biochemical ones.

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